[0016] The present disclosure also relates to a kit comprising the nucleic acid construct, expression vector, host cell, viral particle, or pharmaceutical composition as described above, in one or more containers, optionally further comprising instructions or packaging materials.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. **1**: Immuno fluorescence microscopy images of Huh7 cells transfected with plasmids expressing the indicated human MDR3 isoforms. Wt: wild type sequence, co: codon-optimized sequence. Nuclei were stained with DAPI.

[0018] FIG. **2**: Confocal microscopy images of Huh7 cells transfected with plasmids expressing the indicated human MDR3 isoforms. For isoforms Bwt, Bco, Cwt, and Cco orthogonal projections are shown to demonstrate cytoplasmic localization of MDR3. wt: wild type sequence, co: codon-optimized sequence. Nuclei were stained with DAPI.

[0019] FIG. **3**: MDR3 expression in liver sections from mice injected with pAAV-MDR3 plasmids via HDI (naked DNA transfer). Three images from one mouse injected with MDR3 isoform Aco are shown (left panels), and both mice injected with MDR3 iso form Awt are shown (right panels).

[0020] FIG. **4**: Bile PC concentration in 3 week-old $Abcb4^{-/-}$ mice treated with AAVAnc80-MDR3-Aco vectors. AAV doses are indicated below the x axis. Males (M) are filled symbols and females (F) are open symbols.

[0021] FIG. **5**: Serum alanine transaminase (ALT) and bile salt (BS) levels in AAVAnc80-MDR3-Aco-treated $Abcb4^{-/-}$ mice. Males are indicated in filled symbols, females in open symbols. Samples were collected at 5 days, 1, 2 and 3 weeks post-treatment. d, days; WT, $Abcb4^{+/+}$ mice; KO, $Abcb4^{-/-}$ mice.

[0022] FIG. **6**: Spleen and liver weights (as a percentage of body weight) of $Abcb4^{-/-}$ mice (KO) treated with AAVAnc80-MDR3-Aco at 2 weeks of age and sacrificed 3 weeks later compared to untreated $ABCB4^{-/-}$ (squares) or wild-type (wt) mice (triangles). Males (M) are indicated in filled symbols, females (F) in open symbols. *, $p<0.05$; ns, not significant.

[0023] FIG. **7**: Sirius Red (A-C) and Masson's Trichrome (D-F) staining of liver in male $Abcb4^{-/-}$ mice (KO) treated at 2 weeks of age with saline (A & D) or AAVAnc80-MDR3-Aco at 1.5×10^{13} (B & E) or 5×10^{13} (C & F) and sacrificed 3 weeks later. (G) Quantification of percent area positive for Sirius Red staining was performed via ImageJ software. *: $p<0.05$; ns: not significant.

[0024] FIG. **8**: Bile PC concentration of $Abcb4^{-/-}$ mice (KO) treated with AAVAnc80-MDR3-Aco at 2 weeks of age and sacrificed 3 weeks later compared to untreated $Abcb4^{-/-}$ (squares) or wt mice (triangles). Males (M) are indicated in filled symbols, females (F) in open symbols. ****, $p<0.0001$; ns, not significant.

[0025] FIG. **9**: Serum biomarkers in $Abcb4^{-/-}$ mice treated with AAV8-MDR3-Aco. Males (M) are indicated in filled symbols, females (F) in open symbols. Weeks since treatment are indicated immediately below the x axis, and AAV doses for each group are indicated below.

[0026] FIG. **10**: Spleen and liver weights (as a percentage of body weight) of $Abcb4^{-/-}$ mice (KO) treated with AAV8-MDR3-Aco and sacrificed 12 weeks later compared to untreated $ABCB4^{-/-}$ (KO) or wild-type (wt) mice. Males (M) are indicated in filled symbols, females (F) in open symbols. ***, $p<0.001$**, $p<0.01$; ns, not significant.

[0027] FIG. **11**: IHC staining with anti-MDR3 antibody of liver sections from $Abcb4^{-/-}$ mice treated with saline (top) or AAV8-MDR3-Aco at 5×10^{13} VG/kg (middle) and harvested one week later. Staining of a wild type mouse (WT) liver section is included as a comparator and positive control (bottom).

[0028] FIG. **12**: Serum biomarker levels for a dose range finding study of AAV8-MDR3-Aco in $Abcb4^{-/-}$ mice. The indicated serum markers were analyzed 1 to 10 weeks after vector administration. Males (M) are indicated in filled symbols, females (F) in open symbols. AAV doses are indicated below the x axis.

[0029] FIG. **13**: A) Serum alkaline phosphatase (ALP), alanine transaminase (ALT), B) aspartate transaminase (AST), and bile salt (BS) levels from $Abcb4^{-/-}$ mice treated at 5 weeks of age. Wild-type (WT) animals are indicated with grey open circles, saline-treated $Abcb4^{-/-}$ mice are indicated with black squares, and AAV-MDR3-Aco-treated $Abcb4^{-/-}$ mice are indicated with black filled circles. Males are shown in graphs on the left and females on the right.

[0030] FIG. **14**: Liver (a) and spleen (b) weights (as a percentage of body weight), bile PC (c), fibrosis (d), and MDR3 protein expression (e) in $Abcb4^{-/-}$ mice treated at 5 weeks of age. Wild-type (WT) animals are indicated with grey diamonds, saline-treated $Abcb4^{-/-}$ mice are indicated with open circles, and AAV-MDR3-Aco-treated $Abcb4^{-/-}$ mice are indicated with black triangles.

DETAILED DESCRIPTION

[0031] The invention relates to a transgene comprising a codon-optimized sequence encoding MDR3 isoform A (NCBI reference sequence: NP 000434.1). The membrane-associated protein encoded by ABCB4 gene, also named MDR3 gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. This gene encodes a full transporter and member of the p-glycoprotein family of membrane proteins with phosphatidylcholine as its substrate which may be involved in transport of phospholipids from liver hepatocytes into bile. Alternative splicing of this gene results in three potential isoforms, designated A, B and C.

[0032] As used herein, the term "transgene" refers to exogenous DNA or cDNA encoding a gene product. The gene product may be an RNA, peptide or protein. In addition to the coding region for the gene product, the transgene may include or be associated with one or more elements to facilitate or enhance expression, such as a promoter, enhancer(s), response element(s), reporter element(s), insulator element(s), polyadenylation signal(s) and/or other functional elements. Embodiments of the invention may utilize any known suitable promoter, enhancer(s), response element (s), reporter element(s), insulator element(s), polyadenylation signal(s) and/or other functional elements. Suitable elements and sequences will be well known to those skilled in the art.

Nucleic Acid Construct

[0033] More particularly, the invention relates to a nucleic acid construct comprising a transgene encoding MDR3 isoform A, said transgene being represented by SEQ ID NO: 1 or 2 or having at least 90% identity with SEQ ID NO: 1 or 2.

[0034] The terms "nucleic acid sequence" and "nucleotide sequence" may be used interchangeably to refer to any